Supplemental Oxygen Affects Plasma Insulin-Like Growth Factors in Embryos from Selected Lines of Turkeys[1]

V. L. CHRISTENSEN,*[,2] J. P. McMURTRY,† W. E. DONALDSON,* and K. E. NESTOR‡

*Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7608, †USDA, ARS, Livestock and Poultry Science Institute, Nonruminant Animal Nutrition Laboratory, Beltsville, Maryland 20705, and ‡Department of Poultry Science, The Ohio State University, Ohio Agriculture and Development Center, Wooster, Ohio 44691

ABSTRACT Recent advances in our understanding of insulin-like growth factors (IGF) have improved our knowledge of the physiological roles of these peptide hormones during avian embryogenesis. However, little is known about changes in plasma IGF in response to changes in environmental factors. The objective of the studies reported herein was to examine the response of IGF-I and IGF-II in turkey embryos to changes in incubator gaseous conditions. Two experiments were conducted in which the fractional percentage of oxygen in the incubation atmosphere, a factor known to influence the energy metabolism of embryos, was investigated for its effects on circulating IGF-I and IGF-II in developing turkey embryos.

Oxygenation during pipping and hatching is known to depress lactate, urates, and β-hydroxybutyrate in growth-selected poult embryos, but elevate them in randombred control poult embryos. Plasma concentrations of IGF-II were similarly depressed in the growth-selected hatchlings. Circulating growth factor concentrations were influenced by oxygenation in lines of turkeys in which greater oxygen concentrations enhanced cardiac growth. Enhanced cardiac growth was inversely related to IGF-I concentrations in those genetic lines of turkeys.

It was concluded that changes in poult embryo energy balance as well as changes in growth to adapt to environmental incubator conditions may involve changes in IGF-I and IGF-II. These changes appeared dependent on the genetics of embryos; embryos selected for growth show more fluctuation in response to environmental oxygen than embryos selected for egg production.

(*Key words*: insulin-like growth factors, embryos, turkeys, energy balance, hatching)

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INTRODUCTION

Prior studies with genetic lines of turkeys selected for rapid growth or increased egg production showed that environmental oxygen played a vital role in embryo survival of the randombred control lines but did not improve the survival of either selected line (Christensen *et al.*, 1997). Further investigation indicated that the inability of embryos from the selected lines to respond to supplemental oxygen had a basis in carbohydrate metabolism of the embryo. In particular, embryos from the growth-selected line did not respond with increased gluconeogenic activity when experiencing hypoxia, but the randombred control line embryos did respond. It was speculated and later shown that lactate recycling as well as deamination of gluconeogenic amino acids were depressed following selection for growth, and metabolic ketosis appeared to be increased. The failure of such embryos to respond appropriately to oxygen may have its basis in the physiology of metabolism. The observation that urates and plasma free fatty acids as well as other metabolites were altered following injection of insulin-like growth factor (IGF)-I (McMurtry *et al.*, 1996a) and IGF-II (McMurtry *et al.*, 1998a) into chickens suggested that the growth factors may play a role in the embryonic metabolism of growth-selected turkeys.

The hypothesis proposed by the current study was that IGF in the developing turkey embryo are involved with the hatching process during the hypoxia that normally accompanies the hatching response. The period of hypoxia has become known at the plateau and pipping stages of development (Rahn, 1981). The first objective

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[2]To whom correspondence should be addressed: vern_christensen@ncsu.edu

Abbreviation Key: E = line selected for increased egg production; F = line selected for rapid growth to 16 wk of age; IGF = insulin-like growth factor; NOX = no supplemental oxygen; OX = supplemental oxygen; RBC1 = randombred control line from which E was selected; RBC2 = randombred control line from which F was selected.

was to monitor plasma glucose and IGF-I and IGF-II concentrations in developing turkey embryos when the embryos were exposed to supplemental oxygen at the plateau stage in oxygen consumption, a treatment that has been shown to affect the livability and metabolism of developing turkey embryos (Christensen *et al.*, 1997). The second objective was to monitor differences that might have occurred in plasma growth factor concentrations because of genetic selection for rapid growth or increased egg production in comparison with randombred control lines.

MATERIALS AND METHODS

The materials and methods for supplementing oxygen to turkey embryos throughout the plateau and pipping stages of development in the current experiment have been described in detail elsewhere (Christensen *et al.*, 1997). Fertile eggs from four genetic lines were incubated in commercial incubators until the onset of the plateau stage in oxygen consumption. At the completion of 24 d of incubation, the eggs were randomized and placed into two machines that were operated identically except that one machine received supplemental oxygen (OX) (a partial pressure of 171 ± 3 mm Hg), whereas the other operated under ambient oxygen tensions (NOX) (a partial pressure of 152 ± 3 mm Hg).

The genetic lines used in the current experiments were described elsewhere (Nestor and Noble, 1995). The eggs were from lines selected for rapid growth to 16 wk of age (F line) and its randombred control (RBC2) as well as from lines selected for increased egg production (E line) and its randombred control (RBC1). The breeding stock was reared, and fertile eggs were produced as previously reported (Christensen *et al.*, 1993). Three identical, independent replicate trials of the study were conducted.

Fertile eggs with viable embryos were observed using a candling light to visualize the stage of development beginning at the 25th d of incubation. The time of embryo sampling was determined by observing 24 randomly chosen viable embryos using the candling light. When greater than half of the embryos within a treatment group attained the stage of interest, that treatment combination was sampled. Three embryos were selected randomly immediately prior to pipping, at internal and external pipping, and at hatching (a total of nine per treatment combination across all three trials). The embryo was decapitated and blood was collected into a 10-mL vial contain 10 mg EDTA and placed immediately on ice. The vial was centrifuged ($700 \times g$) for 10 min, and the plasma was decanted into storage vials and frozen until analyzed for IGF-I and IGF-II by radioimmunoassay (McMurtry *et al.*, 1994; McMurtry *et al.*, 1998b, respectively).

Data were sorted by stage of development prior to analysis. The data within a stage of development were arranged in a completely random design as a 2×2 factorial arrangement of treatments. The F line was compared only with its randombred control. The E-line embryos were compared only with RBC1 embryos as the first factor, and the treatments compared with the control were the second factor in the analysis. Probability of a significant difference was based on $P \leq 0.05$. Means determined to differ significantly were separated using the least squares means procedure of the SAS program for personal computers (SAS Institute, 1989).

RESULTS

Oxygen Treatments

The effects of OX on blood concentrations of IGF-I are shown in Tables 1 and 2. The OX treatment elevated IGF-I in both lines at external pipping (Table 1). At hatching, NOX increased IGF-I in the control line compared with the selected line. In the E line and its control (Table 2), there were significant treatment effects at internal pipping and hatching. The OX treatment depressed IGF-I in the E line at internal pipping and hatching, whereas, in the controls, the values did not differ (internal pipping) or differed minimally (hatching). At hatching, OX depressed IGF-I concentrations in both genetic lines.

Plasma concentrations of IGF-II of F and RBC2 embryos were lower in the OX treatment compared with the NOX treatment prior to pipping (Table 3) and at all subsequent stages. The values measured at external pipping did not differ. These differences were not apparent in the E line (Table 4). However, at internal pipping, OX interacted with lines to elevate IGF-II concentrations in E but not RBC1 embryos. No significant differences were found in either genetic line comparison for blood glucose concentrations caused by oxygen or its interaction with genetic line.

Genetic Treatments

In the F line and its control (Table 1), there were no overall differences between the lines for IGF-I plasma concentrations until hatching when RBC values were greater than F. In the E line and its control (Table 2), plasma IGF-I concentrations were decreased prior to pipping in the selected line embryos compared with controls. The plasma IGF-II concentrations did not differ between the F line and its control at any stage of development, but the plasma concentrations of IGF-II were significantly elevated in E embryos at hatching compared with the RBC1.

DISCUSSION

The evidence from the present study suggests the existence of an environmental influence on IGF-I and IGF-II in developing turkey embryos. The factor suggested is oxygen availability. To the authors' knowledge, this is the first report of an environmental factor affecting IGF-I or IGF-II plasma concentrations in turkey embryos. The data also indicate that genetic factors play a role in the endocrine response an embryo makes to oxygenation during the plateau and pipping stages of development.

TABLE 1. Mean blood plasma insulin-like growth factor (IGF)-I concentrations in turkey embryos (n = 9) selected for growth (F) compared with randombred controls (RBC2) when exposed to supplemental oxygen

Genetic line[1]	Treatment[2]	Stage of development			
		Prepip	Internal pip	External pip	Hatchling
		(ng/mL)			
F	NOX	1.4	1.8	1.6	0.5[b]
	OX	2.1	2.6	2.2	1.7[ab]
	$\bar{x}$	1.7	2.2	1.9	1.1
RBC2	NOX	1.7	2.0	2.5	2.2[a]
	OX	1.6	1.9	3.3	1.1[ab]
	$\bar{x}$	1.6	1.9	2.9	1.7
		P			
Line (L)		NS	NS	NS	NS
Treatment (T)		NS	NS	0.05	NS
L × T		NS	NS	NS	0.05
$\bar{x}$ ± SEM (n = 36)		1.7 ± 0.1	2.1 ± 0.2	2.4 ± 0.2	1.4 ± 0.2

[a,b]Column means with no common superscript differ ($P \leq 0.05$).

[1]F = line of turkeys selected for increased 16 wk BW; RBC2 = randombred control population from which the F line was selected.

[2]OX = eggs were incubated with 171 mm Hg of oxygen during 24 to 28 d of incubation; NOX = eggs were incubated with 152 mm Hg of oxygen during 24 to 28 d of incubation.

Oxygen Treatments

In previous studies with the same genetic lines of turkeys, it was shown that oxygen enrichment affected glycogen metabolism and growth (Christensen *et al.*, 1997). Oxygen was shown to affect plasma lactate, urate, and β-hydroxybutyrate levels differently in embryos from different lines as well (Christensen *et al.*, 1998). Observations of the F line selected for increased growth when compared with its randombred control line indicated that OX increased hepatic glycogen in pipping randombred embryos but failed to elicit a response in F embryos. This difference may be related to the observation in the current study that oxygen elevated IGF-II at both internal and external pipping. Oxygen seemed to influence the growth of heart and muscle tissue in E- and RBC1-line embryos more than it did glycogen metabolism (Christensen *et al.*, 1997), and OX in the current study depressed the plasma concentration of IGF-I, suggesting a possible connection between these two events. A connection of IGF-I and IGF-II with oxygen treatments is also suggested by the observations of blood plasma organic acid concentrations under similar conditions (Christensen *et al.*, 1998). Plasma lactate concentrations are strongly affected by the interaction of oxygen treatment and genetics. Oxygen depressed lactate, urate, and β-hydroxybutyrate concentrations in F embryos while elevating them in RBC2 embryos, which suggests a connection between elevated IGF-II concentra-

TABLE 2. Mean blood plasma insulin-like growth factor (IGF)-I concentrations in turkey embryos (n = 9) selected for egg production (E) compared with randombred controls (RBC1) when exposed to supplemental oxygen

Genetic line[1]	Treatment[2]	Stage of development			
		Prepip	Internal pip	External pip	Hatchling
		(ng/mL)			
F	NOX	0.8	3.3[a]	2.6	2.7
	OX	1.9	0.6[b]	1.7	1.2
	$\bar{x}$	1.3[b]	2.0	2.2	2.0
RBC1	NOX	2.5	1.8[ab]	1.0	2.4
	OX	2.1	1.8[ab]	1.8	2.2
	$\bar{x}$	2.3[a]	1.8	1.4	2.3
		P			
Line (L)		0.05	NS	NS	NS
Treatment (T)		NS	0.05	NS	0.05
L × T		NS	0.05	NS	NS
$\bar{x}$ ± SEM (n = 36)		1.8 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	2.1 ± 0.1

[a,b]Column means with no common superscript differ ($P \leq 0.05$).

[1]E = line of turkeys selected for increased 180-d egg production; RBC1 = randombred control line from which E was selected.

[2]OX = eggs were incubated with 171 mm Hg of oxygen during 24 to 28 d of incubation; NOX = eggs were incubated with 152 mm Hg of oxygen during 24 to 28 d of incubation.

TABLE 3. Mean blood plasma insulin-like growth factor (IGF)-II concentrations in turkey embryos (n = 9) selected for growth (F) compared with randombred controls (RBC2) when exposed to supplemental oxygen

Genetic line[1]	Treatment[2]	Stage of development			
		Prepip	Internal pip	External pip	Hatchling
		(ng/mL)			
F	NOX	18.7	8.1	20.3	9.9
	OX	1.0	10.3	32.9	28.7
	$\bar{x}$	9.9	9.2	26.6	19.3[b]
RBC2	NOX	23.9	11.9	14.0	18.4
	OX	11.0	22.7	37.0	28.7
	$\bar{x}$	17.5	17.3	25.5	23.6[a]
		P			
Line (L)		NS	NS	NS	0.05
Treatment (T)		0.05	NS	0.0001	0.01
L × T		NS	NS	NS	NS
$\bar{x}$ ± SEM (n = 36)		13.7 ± 2.0	13.3 ± 1.8	26.1 ± 2.0	21.5 ± 1.4

[a,b]Column followed by different superscript differ ($P \leq 0.05$).

[1]F = line of turkeys selected for increased 16 wk BW; RBC2 = randombred control line from which F was selected.

[2]OX = eggs were incubated with 171 mm Hg of oxygen during 24 to 28 d of incubation; NOX = eggs were incubated with 152 mm Hg of oxygen during 24 to 28 d of incubation.

tions and plasma organic acids involved in energy metabolism and may be similar to those seen previously in broiler chickens (McMurtry *et al.*, 1998a). The E and RBC1 embryos did not change IGF concentrations in response to changes in incubator oxygen as readily as did the F and RBC2 lines, but the data still suggest a relationship between IGF-I and IGF-II and blood lactate, urate, and β-hydroxybutyrate concentrations. Elevated lactate, urate, and β-hydroxybutyrate concentrations observed in previous studies (Christensen *et al.*, 1997) may be related to changes observed in IGF-I and IGF-II concentrations, even though, in E and RBC1 embryos, the effects appeared to be related to growth more than to glycogen metabolism. The IGF effects were also noted in response to environmental oxygen concentration. Little is known of the environmental influences that may affect IGF-I or IGF-II levels in turkey embryos.

Genetic Effects

The ontogeny of IGF-I and IGF-II has been reported recently in the turkey embryo as well as in extra-embryonic membranes of the developing turkey embryo (McMurtry and Brocht, 1997). The IGF play crucial roles in embryonic and postnatal development, differentiation, and growth of turkeys (Daughaday and Rotwein, 1989; DePablo *et al.*, 1991; McMurtry and Brocht, 1997). Insulin-like growth factor is expressed in the turkey liver under

TABLE 4. Mean blood plasma insulin-like growth factor (IGF)-II concentrations in turkey embryos (n = 9) selected for egg production (E) compared with randombred controls (RBC2) when exposed to supplemental oxygen

Genetic line[1]	Treatment[2]	Stage of development			
		Prepip	Internal pip	External pip	Hatchling
		(ng/mL)			
E	NOX	15.8	22.3[a]	18.6	23.5
	OX	16.5	7.6[b]	20.9	25.6
	$\bar{x}$	16.2	15.0	19.7	24.6[a]
RBC1	NOX	10.8	14.8[ab]	23.5	15.1
	OX	11.7	12.3[ab]	26.2	16.4
	$\bar{x}$	11.3	13.6	24.9	15.8[b]
		P			
Line (L)		NS	NS	NS	0.05
Treatment (T)		NS	NS	NS	NS
L × T		NS	0.05	NS	NS
$\bar{x}$ ± SEM (n = 36)		13.7 ± 2.0	14.3 ± 1.4	22.3 ± 2.1	20.1 ± 2.0

[a,b]Column means with no common superscript differ ($P \leq 0.05$).

[1]E = line of turkeys selected for increased 180-d egg production; RBC1 = randombred control line from which E was selected.

[2]OX = eggs were incubated with 171 mm Hg of oxygen during 24 to 28 d of incubation; NOX = eggs were incubated with 152 mm Hg of oxygen during 24 to 28 d of incubation.

the regulation of growth hormone (GH) (McMurtry *et al.*, 1998b), whereas the expression is thought to be GH independent in other embryonic tissues (Rosselot *et al.*, 1995). McMurtry *et al.* (1996b) have recently reported that in the developing turkey embryo, IGF-I can be detected in the circulation on Day 10 of development, IGF-II can be detected at Day 12 of incubation (McMurtry and Brocht, 1997), and levels peak during mid-embryogenesis. The source of IGF-I and IGF-II in turkey embryos is unknown. In a recent study, McMurtry *et al.* (1998a) injected chicken and human IGF-II to determine endocrine and metabolic effects on 5-wk-old broiler chickens. They observed significant depressions in plasma insulin, GH, thyroxine, and triiodothyronine concentrations. Additionally, glucagon, uric acid, calcium, and plasma free fatty acids were elevated. The results of the current study imply that selection for growth may make embryos respond to changes in environmental oxygen, and, further, that these changes may be associated with gluconeogenic processes, affecting plasma lactate and urate concentrations (Christensen *et al.*, 1999). Selection for egg production has caused embryos to respond differently to hypoxia than those selected for growth. Response to oxygenation for these lines (E and RBC1) was mostly in cardiac growth (Christensen *et al.*, 1997), suggesting that a more critical response for this genetic line may be IGF influences on tissue growth. Cardiac growth because of IGF-I has recently been noted in rats as well (Donath *et al.*, 1998).

Summary and Conclusions

Data in the current study suggest a physiological role for IGF in carbohydrate metabolism of turkey embryos. When embryos were exposed to OX during pipping and hatching, IGF-I and IGF-II plasma concentrations were elevated in a stage- and strain-dependent manner. Additionally, IGF-I and IGF-II plasma concentrations differed between selected lines and their controls, suggesting a genetic component for IGF function in turkeys.

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